Workshop Concept: Exposure to Permanent Hair Dyes and Cancer: Needs and Approaches for Improved Hazard Characterization

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1. Rationale and Goals

Millions of people in the United States and Europe use hair dyes, mostly permanent hair dyes. In the late 1970s some hair dyes were taken off the market because they caused cancer in laboratory animals or were mutagenic. Some hair dyes on the market today are in the same chemical class as those banned. Associations between exposure to hair dyes and urinary bladder cancer and non-Hodgkin's lymphoma (NHL) have been reported in some epidemiological studies; however, the available studies are not able to resolve whether hair dye use in humans is a cancer hazard, and if so, which dyes are carcinogenic. There are no specific guidelines in the United States for testing individual hair dyes or commercial hair colorants. Given the widespread use of permanent hair dyes, the possibility that hair dyes cause cancer is a high public health concern.

The NTP proposes a workshop on hair-dye use and cancer to improve hazard characterization. This workshop should provide a forum for a focused, cross-disciplinary discussion on the path forward to answer questions about the potential carcinogenicity of permanent hair dyes. It should also provide an opportunity to enhance communication among stakeholders and foster future interactions. The goals of the workshop are to:

- identify the available scientific information regarding the carcinogenicity of hair dyes
- identify knowledge gaps in testing and scientific research evaluating hair dyes and cancer risk
- identify approaches to improve (1) toxicity testing of individual dyes and/or commercial products, and (2) epidemiologic studies
- identify and propose potential mechanisms of cancer induction and research strategies to test them
- foster multi-disciplinary discussions to facilitate the use of toxicology to inform epidemiologic study design

2. Background Information

In the United States and Europe, it is estimated that more than one-third of women over age 18 and about 10 percent of men over age 40 use some type of hair dye (Huncharek and Kupelnick 2005). Hair dyes are classified into three major groups: temporary, semi-permanent, and permanent hair colors. Permanent (or oxidative) hair dyes, which account for 70% to 80% of the coloring product market, are the result of a chemical reaction between a primary intermediate (colorless dye) and a coupler. The reactants are usually aromatic amines and phenolic compounds. In the presence of a developer (such as hydrogen peroxide) under aqueous, basic pH conditions, the intermediate is oxidized and rapidly reacts with a coupler(s) resulting in colored aromatic reaction products. Darker colors are formed by using higher concentrations of intermediates. During the dyeing process, which usually lasts 30 minutes, the consumer is exposed dermally to primary intermediate(s), coupler(s), and reaction product(s) as well as other agents, such as ammonia, peroxide, fragrance, and conditioners (Corbett, 2000, Zviak and Milléquant, 2005).

2.1. Safety Testing in the United States and Europe

Hair colorants used in the United States and the European Union are under different regulations and safety assessment procedures. In the United States, no pre-market approval is required by the Food and Drug Administration (FDA) for cosmetics or their ingredients, with the exception of some color additives. Color additives, which are intended for use as coal tar hair dyes (dyes derived from petroleum products), are not subject to pre-market approval, providing the product is labeled with a prescribed caution statement[1]. The U.S. Food, Drug, and Cosmetic Act requires, prior to marketing, that every cosmetic and personal care product and its ingredients be safe under labeled or customary conditions of use and that they contain no prohibited ingredients (FDA 1997, 2005). Safety assessments are performed by the Cosmetic Ingredient Review (CIR) board, an independent panel of experts financially supported by the Personal Care Products Council (CIR 2011). CIR assessments as well as data from other sources provide product safety information to the FDA. The FDA has post-market authority over cosmetics and can remove or restrict unsafe or misbranded products. In the European Union, the Scientific Committee on Consumer Products (SCCP) conducts safety evaluations on hair dye products, and provides a list of approved and banned hair dye chemicals for the European market in accordance with a published assessment strategy (EU 2005, 2010).

2.2. Studies of Hair Dyes and Cancer

Safety concerns over the use of hair dyes have a long history with reports in the news media dating back over a century (The New York Times 1883, 1908, 1909). Aromatic amines are of concern because this chemical class causes chemically induced neoplasms in experimental animals, primarily in the liver and urinary tract (Miller *et al.* 1964). Metabolism of aromatic amines can occur through Phase I and/or Phase II enzymes and, for example, can be harmful through formation of a nitrenium ion or protective through acetylation of the amine group. Electrophilic ions can react with DNA, form adducts, and lead to point mutations (Klaunig and Kamendulis, 2008; IARC 2010a). *para*-Phenylenediamine (PPD), a major aromatic amine used in the formulation of hair dyes, has been shown to induce DNA damage in cultured human urothelial cells (Huang *et al.* 2007). However, whether or not the chemically induced DNA damage occurs may be explained by individual differences in metabolism of aromatic amines (IARC 2010a).

2.2.1. Studies in Experimental Systems

The National Cancer Institute (NCI)/National Toxicology Program (NTP) in 2-year bioassays has tested 26 chemicals used in hair dyes by administration in the diet or by gavage in rats and mice and one chemical by dermal administration. A few of these dyes are listed as *reasonably anticipated to be a human carcinogen* in the Report on Carcinogens and as *possibly carcinogenic to humans* (group 2B) by International Agency for Research on Cancer (IARC1993). In 2008, an IARC Working Group concluded that there was a pressing need for well-designed animal carcinogenicity studies, as studies of hair color formulations were limited by poor study design and lack of reporting of some toxicity endpoints, small numbers of animals, and use of concentrations well below a maximum tolerated dose (IARC 2010b).

2.2.2. Human Epidemiological Studies

The 2008 IARC Working Group (2010b) also recently concluded that there was inadequate evidence for the carcinogenicity of personal use of hair dyes from studies in humans. The two

[1] "Caution-This product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should first be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness." [FD&C Act, section 601(a)]

cancers of most concern are urinary bladder cancer and NHL. Although some of the five most recent case-control studies (published after the 1993 IARC review) found significant risks for urinary bladder cancer among individuals using permanent hair dyes (Gago-Dominguez *et al*. 2001a,b), other studies have not found a positive association. Findings from studies published since the IARC review have been inconsistent (Mendelsohn *et al.* 2009, Shakhssalim *et al.* 2010).

For NHL, one of the most informative studies is a pooled analysis of three case-control studies that evaluated risks for different subtypes of NHL. Risks from using hair dyes were elevated for follicular lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma, but not for other subtypes of NHL; however, the magnitude of the risk estimates increased only slightly with increasing intensity or duration of use (Zhang *et al*. 2008, IARC 2010b). Some studies have suggested that the increased risk was limited to consumers who started using permanent hair dyes prior to 1980 (Benavente *et al*. 2005, Zhang *et al.* 2009); however, fewer individuals in these studies used hair dyes after the 1980s compared to those using hair dyes prior to the 1980s, and the latency period was shorter. Also, differences in risk of cancer due to the shade of the hair dye also are not clear.

The 2008 IARC Working Group also concluded that there was limited evidence of an increased risk for cancer of the urinary bladder in hairdressers, and meta-analyses reported since that time have supported this conclusion (Takkouche *et al.* 2009, Harling *et al.* 2010). Hairdressers are exposed to many other chemicals in addition to hair dyes, and thus these studies have limited relevance for evaluating cancer risks specific to use of hair dyes.

Some studies of personal hair dye use have reported that polymorphisms in metabolism genes (CYP2C9, CYP2E2, GSTM3, GSTP2, or NAT2) modified the risk of urinary bladder cancer and NHL (Gago-Dominguez *et al*. 2001b, 2003, Zhang *et al*. 2009).

3. Altered Immunity and its Relationship to Hair Dyes and NHL

Altered immunity, which has been associated in some studies with hair-dye use, is a risk factor for NHL. Hair dyes have been shown to affect the immune system, either by activating pro-inflammatory or anti-inflammatory immune responses.

3.1. Altered Immunity as a Risk Factor for NHL

NHL arises from precursor lymphocytes arrested at a specific stage of development as a result of a multi-step process involving accumulation of genetic damage and clonal expansion of malignant T or B cells (Fisher and Fisher 2004). Dysregulation of the immune system, such as chronic antigenic stimulation and immunosuppression, is thought to play a role in the carcinogenic process (Grulich and Vajdic 2005, Grulich *et al.* 2007). Chronic antigenic stimulation promotes B-cell proliferation, which increases the probability of a random or chemically induced genetic mistake; it also leads to down-regulation of the T-cell response, which results in immunosuppression. B-cell tumors are more likely to develop in this scenario. Immunosuppression may promote lymphomagenesis by inhibiting recognition and eradication of the transformed cell (Fisher and Fisher 2004).

3.2. Hair Dyes and Immune Effects

Permanent hair dyes cause allergic contact dermatitis in some consumers. Most patients suffering from adverse allergic skin reactions have a positive patch test reaction to PPD, which is used in more than two-thirds of oxidative hair dyes. Commercial hair dyes containing PPD are potent immune activators and cause dermal sensitization and systemic increases of inflammatory cytokines in animal models (Bonefeld *et al.* 2010).

In contrast to animal models in which all animals developed allergic reactions, only a minority of consumers exposed to permanent hair dyes develop contact dermatitis, suggesting that the majority of individuals do not become sensitized or develop immune tolerance (Rubin *et al.* 2010). However, one of the limitations of some animal models is that animals are usually exposed to hair dyes on a daily basis, whereas exposure is less frequent for most consumers. Repeated exposure of mice to permanent hair dye in a more consumer-like manner (administered weekly) induced regulatory T cells and interleukin-10, suggesting that hair dyes also induce anti-inflammatory mechanisms.

4. Key issues

- Assessment of the carcinogenicity of hair dyes is complicated by (1) the large numbers (over 100) of individual dyes and other chemicals used in the dyeing process, (2) the variation in the types of dyes across commercial brands and shades of dyes, (3) the changes over time in the types of dyes used, and (4) exposure to a mixture of chemicals in hair dye products, including those formed during the dyeing process.
- There is uncertainty whether the reported excess risks of cancer observed in some epidemiologic studies are limited to exposures to dyes used prior to 1980 or limited to specific shades of hair dyes. A number of agents shown to be genotoxic (Ames *et al.* 1975) or known to cause cancer in experimental animals were removed from the market prior to 1980, and some epidemiologic studies find that elevated risks of NHL are limited to use of permanent hair dyes starting prior to the 1980s.
- The relationship between hair-dye–mediated immune effects and NHL has not been explored and needs evaluation.

5. Proposed Approach

We propose to convene a public workshop in the Research Triangle Park, North Carolina area as a forum for a cross-disciplinary discussion to identify knowledge gaps and propose research recommendations that would improve hazard characterization of hair-dye use and cancer.

5.1. Workshop Planning Committee

The Office of the Report on Carcinogens (ORoC) will form a workshop planning committee of scientists with relevant expertise and knowledge from NIEHS, FDA, other government agencies, and members of the scientific community to provide advice on workshop activities described in Sections 5.2 to 5.4. Specifically, the committee will (1) provide comment on the workshop materials (primarily the literature review document), (2) advise on workshop participants, and (3) develop focused subtopics and specific charge questions for the workshop breakout groups.

5.2. Literature Review Document and Meeting Presentations

Prior to the workshop, the ORoC staff, with input from the planning committee and topic experts, will prepare a draft literature review document that summarizes relevant topics related to hair dye use and cancer, including but not limited to those listed below. The recent IARC monograph (IARC 2010b), which evaluated the state of the science on exposure to hair dyes and cancer, will serve as a valuable resource and starting point for the workshop review document.

- chemistry of hair dyes
- findings from cancer studies in experimental animals and a discussion of the strengths and limitations of the animal models to predict cancer hazard in humans

- information on potential short-term testing systems: e.g., compilation of high-throughput data on some hair dyes tested as part of Tox21 and a summary of the draft European strategies for hair dye testing and list of approved and banned hair dye chemicals (EU 2005, 2010)
- epidemiologic studies evaluating exposure to hair dyes and cancer hazard
- studies evaluating toxicokinetics, genotoxicity, immunotoxicity, and other effects related to carcinogenicity

The meeting will include plenary and breakout group sessions. Oral presentations during plenary sessions will include summaries of the (1) literature on key topics related to hair dyes and cancer, and (2) recommendations from the breakout group deliberations.

5.3. Participants and organization of the workshop

The planning committee will work with ORoC staff to identify workshop participants with relevant expertise such as chemistry, exposure assessment, toxicology, immunology, epidemiology, and statistics. The workshop will be open for attendance by other interested individuals/groups as observers.

Based on a review of the recent IARC monograph, and additional literature searches, ORoC has identified some knowledge gaps and general topics for breakout groups (listed below), but anticipates that these will be refined during planning for the workshop. Workshop participants will also be asked to identify additional research topics and recommend research and testing strategies. The workshop planning committee will develop focused subtopics and specific charge questions for each of the breakout groups. The meeting will be organized to allow for multiple, sequential opportunities for dialogue across breakout groups with the objective of developing recommendations that are based on cross-disciplinary discussions.

Proposed key topics for breakout groups include:

- Recommendations for toxicity testing of individual dyes and/or commercial products

  Breakout group discussions will include identifying and improving short-term and long-term testing strategies (e.g., cancer bioassays, dermal absorption and metabolism studies, genotoxicity assays, high throughput screening [Tox21], gene expression assays, or other short-term assays).

- Identification and proposal of potential mechanisms of cancer potentially applicable for hair dyes, and development of research strategies to evaluate them

  An example of a proposed breakout group topic is mechanistic studies to characterize hair dye–mediated immune effects as they relate to cancer development, focusing on NHL. The research strategies should include information on the chemical(s) (e.g., individual dyes or commercial products), endpoints to be tested, and appropriate experimental models.

- Identification of biomarkers of exposure, early effects, and genetic susceptibility to be used in epidemiologic studies
  - Exposure markers – focus on absorption and metabolism
  - Early effects – such as immune responses and genotoxic effects
- Recommendations for improving epidemiologic studies

Breakout group discussions will include (1) the use of toxicological data such as the incorporation of biomarkers of exposure, effects, genetic susceptibility, and knowledge from mechanistic studies to inform epidemiologic design, (2) improving exposure assessment and identifying the most relevant exposure metrics, and (3) facilitating collaborations between investigators to increase study size.

Many women using or applying hair dyes are of childbearing age; thus, there is also a need to evaluate potential reproductive and developmental effects. However, broadening the scope of the workshop would limit the capability for a focused, cross-disciplinary discussion on hair dyes and cancer. In addition, there is a limited database on reproductive and developmental effects and exposure to hair dyes. Nevertheless, some of the background information in the literature review documents and recommendations from this workshop, such as absorption and metabolism data, toxicity testing strategies, and improving epidemiologic study design, will be meaningful for studying other health effects.

5.4. Workshop products

The NTP plans to publish a final workshop report containing the literature summaries, the opinions and recommendations of the breakout groups, and overall workshop synopses that will be published on the NTP website, and possibly published in the peer-reviewed literature.

6. Significance and Expected Outcomes

Many studies have evaluated the relationship between exposure to permanent hair dyes and cancer; however, the question whether hair-dye use in humans is a cancer hazard is still unresolved. This workshop offers a unique opportunity to bring together scientists with expertise in multiple disciplines (such as exposure assessment, epidemiology, toxicology, and immunology) for a focused discussion of the path forward to help answer this question. The open dialogue of a workshop format should facilitate the exchange and sharing of ideas among participants and stimulate innovative research and testing activities, collaboration, and possibly hazard evaluations. The final workshop report and any related publications will be a useful resource to the NTP, NIEHS, other government agencies, research organizations, and the scientific community.

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